

Amendments to the Title:

At page 1, please amend the title as follows:

NUCLEIC ACIDS ENCODING A GALACTURONOSYL TRANSFERASES,
(GALAT1) ENZYME FROM ARABIDOPSIS ~~NUCLEIC ACIDS ENCODING SAME AND~~
~~USES THEREFOR~~

Amendments to the Specification:

At page 8, please rewrite lines 27-29, as follows:

Fig. 3 shows the representative structure for rhamnogalacturonan II (RG-II). RG-II has a backbone of 1,4-linked alpha-D-GalpA residues. GalA residues are also present in RG-II side chain A. Other side chains are denoted B, C and D.

At page 9, please rewrite lines 24-31 as follows:

Fig. 8 demonstrates that recombinant JS36 (At3g61130) has galacturonosyltransferase (GalAT) activity. Human embryonic kidney cells (HEK293) were transiently transfected with the pEAK vector alone, or with pEAK vector containing the truncated versions of JS33 or JS36. Total media (1); protein immunoabsorbed from the medium using anti-HA epitope:Protein A ~~Sepharose~~ SEPHAROSE (2); and protein immunoabsorbed from the medium using anti-HA epitope:Protein G ~~Sepharose~~ SEPHAROSE (3) were tested for GalAT activity. Data are the average [¹⁴C]GalA incorporated into product from duplicate reactions from three separate experiments.

At pages 14-15, bridging paragraph, please rewrite as follows:

In order to identify a gene(s) involved in pectin biosynthesis, the inventors used a partial purification-tandem mass spectrometry approach to identify putative *GALAT* genes from *Arabidopsis* (see Fig. 5 for strategy). GalAT from *Arabidopsis* was partially purified from detergent-solubilized enzyme by sequential passage over two or more of the following resins: cation exchange resin ~~SP-Sepharose~~ SP-SEPHAROSE, reactive green 19 resin, reactive blue 72 resin, reactive yellow 3 resin, and UDP-agarose. Proteins obtained from selected fractions from these columns were treated with trypsin to generate peptides, and the amino acid sequence of the peptides identified by liquid

chromatography-tandem mass spectrometry. The amino sequence thus generated was used to screen the *Arabidopsis* gene/protein database. Thirty unique proteins were solely identified in the GalAT-containing fractions (i.e. not present in fractions not containing GalAT activity). Among the 30 unique proteins that co-purified with GalAT activity, two proteins (designated JS33 and JS36) were initially identified as *Arabidopsis* putative GALAT proteins/genes based on their having at least one predicted transmembrane domain and since they contained a predicted glycosyltransferase domain (see CAZy database; ~~<http://afmb.cnrs.mrc.fr/CAZY/index.html>~~ website available as afmb.cnrs.mrc.fr/CASY/index.html).

At page 15, heading of Table III, lines 27-30, please rewrite as follows:

Table III. Predicted characteristics of JS36, JS33 and JS36L proteins. Predictions were made using information from the NCBI database and the SOSUI (Classic & Membrane Prediction program) at BCM Search Launcher site (~~<http://searchlauncher.bcm.tmc.edu/seq-search/struc-predict.html>~~) (searchlauncher.bcm.tmc.edu/seq-search/struc-predict.html).

At page 16, please rewrite the first paragraph, lines 1-9, as follows:

The truncated forms of JS33, JS36 and JS36L, and the vector alone, were transiently expressed in human embryonic kidney cells (HEK293 cells) for 46 hours. Since the translational fusion proteins constructed contained two copies of the HA epitope, the culture medium was collected and a portion was treated with a mouse anti-HA IgG1 bound either to Protein A ~~Sepharose~~ SEPHAROSE or Protein G ~~Sepharose~~ SEPHAROSE. The immunoadsorbed protein was assayed for GalAT activity using UDP-[¹⁴C]GalA and a mixture of OGA acceptors. Figure 8 shows that the JS36 construct expressed a protein exhibiting GalAT activity. These studies establish that JS36 is a GalAT and thus we designated the gene GALAT1.

At page 16, please rewrite the third paragraph, lines 20-28, as follows:

GALAT1 is a member of the Glycosyltransferase Family 8 in the CAZy database [database of putative and proven carbohydrate modifying enzymes that currently contains 61 different proposed glycosyltransferase families (<http://afmb.cnrc-mrc.fr/CAZY/index.html>) website is afmb.cnrc-mrc.fr/CAZY/index/html] 66,67]. The presence of GALAT1 in Family 8 is in agreement with our demonstrated activity of GALAT1 as an α 1,4-galacturonosyltransferase, since Family 8 is a family of proposed retaining glycosyltransferases and GALAT1 is a retaining enzyme, i.e., the α -configuration in the substrate UDP- α -GalA is retained in the product α 1,4-linked-galacturononan (HGA).

At page 16, please rewrite the last paragraph, lines 30-35, as follows:

GALAT is expressed in multiple Arabidopsis tissues at multiple times during development. We base this on our RT-PCR analysis of RNA from Arabidopsis flower, root, stem and leaf tissue (Figs. 6A and 6B) showing that GALAT1 is expressed in all these tissues, and based on the 18 EST entries for this gene in the TAIR database (<http://www.arabidopsis.org>) (website entitled [arabidopsis.org](http://www.arabidopsis.org)) indicating that GALAT1 is expressed in developing seed, green siliques, roots and above ground organs.

At page 17, please rewrite the second paragraph, lines 14-34, as follows:

Mutant studies provide further evidence that the GalAT family encodes GalATs involved in pectin synthesis. We recently used seed received from Arabidopsis T-DNA mutant collection (SIGNAL; <http://signal.salk.edu/cgi-bin/tdnaexpress> website entitled signal.salk.edu/cgi-bin/tdnaexpress) to identify and generate six homozygous Arabidopsis GalAT family T-DNA insert mutant lines of several members of the GalAT family. We found that one GalAT family gene At1g06780, when mutated, produces

leaves with cell walls that contain reduced amounts of galacturonic acid. Specifically, analysis of walls from homozygous mutant line 073484 revealed that the walls had an 18% reduction in GalA and a concomitant increase in glucose. None of the other sugars changed. Of the three available At1g06780 T-DNA insert lines, no homozygous seed was recovered from mutants where the T-DNA was inserted into an exon. Rather, seed recovered from such lines had a reduced germination rate. In line 073484, however, the T-DNA is inserted in the 5'-UTR, suggesting that it may have a leaky phenotype. The results are consistent with gene At1g06780 encoding a GalAT and with the identification of the gene family as a GalAT gene family. The GalA content of the walls of another Arabidopsis mutant (Quasimodo) is reduced by 25% and these plants exhibit decreased cell adhesion⁵⁵, characteristics consistent with the Quasimodo gene encoding a GalAT. Quasimodo has 53% amino acid identity and 72% similarity to GALAT1 and the gene affected in Quasimodo (At3g25140) is a member of our proposed GalAT family. There is, however, at present no direct enzymatic evidence that the protein encoded by Quasimodo is a functional GalAT.

At page 22, please rewrite the second full paragraph, lines 23-33, as follows:

Characteristics of the recombinant truncated GALAT1 can be compared to the GALAT1 solubilized from Arabidopsis membranes by immunoabsorption of the solubilized GALAT1 using anti-GALAT1 antibody (see section below) bound to Protein A or G ~~Sepharose~~ SEPHAROSE, or by coupling the anti-GALAT1 antibodies to 3M-Emphaze ~~resin~~ ⁶⁶ resin and using the resin used to purify GALAT1 from solubilized Arabidopsis enzyme. If the characteristics of the immunoabsorbed Arabidopsis GALAT1 are different from those of the recombinant truncated GALAT1, the immunoabsorbed GALAT1 can be analyzed by LC tandem mass spectrometry to determine if additional proteins are immunoabsorbed with the Arabidopsis solubilized GALAT1 that may have modified the activity (e.g. a heteromeric complex).

At page 23, please rewrite the first paragraph, lines 1-13, as follows:

The recombinant GALAT1 and the GALAT1 immunoadsorbed-from Arabidopsis solubilized membranes can also be treated with N-glycanase to determine if they are N-glycosylated. To determine if they are O-glycosylated, the proteins can be exhaustively treated with N-glycanase, the released oligosaccharides removed, and the resulting protein analyzed by TMS methylation analysis to determine the glycosyl residue composition of any carbohydrates still attached to the protein. Any oligosaccharide released by the N-glycanase treatment can also be analyzed by TMS methylation. The results of these experiments would indicate whether the native Arabidopsis GalAT is glycosylated and whether the recombinant forms have the same or different glycosylation pattern. Changes in glycosylation could affect GalAT1 enzyme activity and/or substrate binding. GALAT1 is predicted to have 5 or 6 N-glycosylation sites (NetNGlyc 1.0 Prediction; <http://www.expasy.org/sitemap.html> — website entitled expasy.org/sitemap.html).

At page 25, please rewrite the first full paragraph, lines 17-29, as follows:

Double-stranded RNA-mediated interference (RNAi) is a method to study the function of genes in plants¹⁰⁰. Transgenic plants harboring an RNAi construct often have reduced expression of the gene-specific mRNA. The resulting plants may display either complete gene silencing, thus having a knockout phenotype, or a partial “knockout” phenotype due to ‘leaky’ expression. The RNAi approach should allow the suppression of GALAT1 expression and a reduction or loss of GALAT1. This enables one to elucidate the function of GALAT in pectin synthesis and in the plant. Simultaneously, the sequence-indexed T-DNA insertion mutants listed in the Salk Institute Genomic Analysis Laboratory (SIGnAL) Arabidopsis T-DNA mutant collection (<http://signal.salk.edu/cgi-bin/tdnaexpress> — website entitled signal.salk.edu/cgi-bin/tdnaexpress) can be monitored to determine if any T-DNA insert lines for GALAT

become available. If so, the seed can be obtained and the mutants generated therefrom can be characterized (as described above).

At pages 26-27, please rewrite the bridging paragraph as follows:

A person of ordinary skill in the art can use mutant seeds to probe gene function. For example, the initial mutant seed (often a segregating T3 line, see ~~(http://signal.salk.edu/tdna_FAQS.html)~~ website entitled signal.salk.edu/tdna_FACs.html) can be grown and selfed to increase the seed stock (T4). Multiple plants from T4 seed can be grown and the presence of, for example) the T-DNA insert determined by PCR of plant genomic DNA using a T-DNA primer and a gene specific primer. The same DNA can be analyzed with gene specific primers that should span the T-DNA insertion site. These analyses should indicate whether the given plant contains a T-DNA insert and if so, whether it is homozygous or heterozygous for the mutation. If necessary, Southern blotting and hybridization with the specific genes can be used to determine if the gene contains the expected T-DNA insert. Seed homozygous for the T-DNA insertion (when not lethal) or heterozygous (when no viable TDNA homozygous plants are obtained) can be selfed to amplify the seed and, for heterozygous plants, to test for segregation of any phenotype or T-DNA insert. Plants can be scored as heterozygous or homozygous by PCR analysis of the T-DNA insert and by any visible phenotype. Homozygous or heterozygous plants can be used for growth phenotype and cell wall analysis. The seed can also be crossed with wild type Columbia and then selfed to eliminate the possibility that the lines contain an unexpected mutation or additional T-DNA insert(s).